

08/16/99



08/16/99

UTILITY PATENT APPLICATION TRANSMITTAL
(only for new and continuation-in-part
nonprovisional applications under 37 CFR
1.53(b))

Docket No.:
P-LJ 3597

Address to: ASSISTANT COMMISSIONER FOR
PATENTS
Box Patent Application
Washington, D.C. 20231

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"EXPRESS MAIL" MAILING LABEL NUMBER: EL 454954871US

DATE OF DEPOSIT: August 16, 1999

I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING
DEPOSITED WITH THE UNITED STATES POSTAL SERVICE
"EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER
37 C.F.R. 1.10 ON THE DATE INDICATED ABOVE, AND IS
ADDRESSED TO: THE ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231.

Robert Gibbons

(TYPED OR PRINTED NAME OR PERSON MAILING PAPER OR FEE)

Robert Gibbons

(SIGNATURE OF PERSON MAILING PAPER OR FEE)

08/16/99

09/375246



08/16/99

This is a request for filing a
X new utility patent application under 37 CFR 1.53(b)(1).
____ continuation-in-part under CFR 1.53(b)(2) of prior
application serial no. _____, filed _____
(list entire parentage).

Title: METHODS FOR IDENTIFYING SOMATIC CHANGES IN GENOMIC
SEQUENCES USEFUL FOR CANCER DIAGNOSIS AND PROGNOSIS

Inventor(s) (full name of each inventor): Manuel Perucho

Enclosed are:

- X Return receipt postcard
- Initial Information Data Sheet
- X 1 Page application cover sheet
- X 41 Pages of specification (includes claims and abstract)
- X 7 Sheets of drawing(s)
- Pages of an executed Declaration for Patent Application
- An executed Power of Attorney for Patent Application by
Assignee
- Paper copy of sequence listing, pages ____ through ____
- Sequence listing in computer readable form
- Statement Under 37 CFR 1.821(f)
- An executed assignment and cover sheet
- An executed small entity statement
- Also enclosed: _____

Inventors: Manuel Perucho
Docket No.: P-LJ 3597
Page 2

— This application is based on prior foreign application(s)
No.(s) _____, filed in _____ on _____
_____, respectively, and priority is hereby claimed therefrom.

X This application is based on, and claims the benefit of, U.S.
Provisional Application No. 60/096,828, filed August 17, 1998,
and entitled METHODS FOR IDENTIFYING TUMOR GENES AND NOVEL
TUMOR GENES.

— This application is based on, and claims the benefit of, U.S.
Provisional Application No. 60/_____ (yet to be assigned),
filed _____, which was converted from U.S. Serial No.
_____, and entitled _____.

The filing fee has been calculated as shown below:

	Number Filed		Number Extra		Rate			Fee	
					Small Entity	Other Entity		Small Entity	Other Entity
Total Claims	23-20	=	3	x	\$9	\$18	=	\$	\$
Indepen- dent Claims	5 - 3	=	2	x	\$39	\$78	=	\$	\$
Multiple Dependent Claims Presented: ___ Yes ___ No					\$130	\$260		\$	\$
					BASIC FEE			\$380	\$760
					TOTAL FEE			\$	\$

— A check in the amount of \$ _____ to cover the filing fee is
enclosed.

X The payment of the filing fee is to be deferred until the
Declaration is filed. Do not charge our deposit account.

— The Commissioner is hereby authorized to charge fees under 37
CFR 1.16 and 1.17 which may be required or credit any
overpayment to Deposit Account No. 03-0370. A duplicate copy
of this sheet is enclosed.

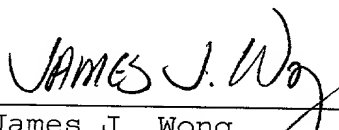
Inventors: Manuel Perucho
Docket No.: P-LJ 3597
Page 3

Address all future communications to:

Cathryn Campbell
CAMPBELL & FLORES LLP
4370 La Jolla Village Drive, Suite 700
San Diego, California 92122
telephone: (858) 535-9001
facsimile: (858) 535-8949

Respectfully submitted,

Date: August 16, 1999



James J. Wong
Registration No. 34,949
CAMPBELL & FLORES LLP
4370 La Jolla Village Drive
Suite 700
San Diego, California 92122

003546 094609
"STAGE" 094609

A P P L I C A T I O N

for

UNITED STATES LETTERS PATENT

on

METHODS FOR IDENTIFYING SOMATIC CHANGES
IN GENOMIC SEQUENCES USEFUL
FOR CANCER DIAGNOSIS AND PROGNOSIS

by

Manuel Perucho and Sergei Malkhosyan

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

"EXPRESS MAIL" MAILING LABEL NUMBER: EL454954871US

DATE OF DEPOSIT: August 16, 1999

I HEREBY CERTIFY THAT THIS PAPER OR FEE IS BEING
DEPOSITED WITH THE UNITED STATES POSTAL SERVICE
"EXPRESS MAIL POST OFFICE TO ADDRESSEE" SERVICE UNDER
37 C.F.R. 1.10 ON THE DATE INDICATED ABOVE, AND IS
ADDRESSED TO: THE ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231.

Robert Gibbons
(TYPED OR PRINTED NAME OR PERSON MAILING PAPER OR FEE)

Robert Gibbons
(SIGNATURE OF PERSON MAILING PAPER OR FEE)

Attorneys

CAMPBELL & FLORES LLP
4370 La Jolla Village Drive, Suite 700
San Diego, California 92122

Sheets of Drawings: Seven
Docket No.: P-LJ 3597

**METHODS FOR IDENTIFYING SOMATIC CHANGES IN GENOMIC
SEQUENCES USEFUL FOR CANCER DIAGNOSIS AND PROGNOSIS**

This application claims the benefit of U.S.
Provisional Application No. 60/096,828, filed
5 August 17, 1998, and is incorporated herein by reference.

This invention was made with government support
under National Institutes of Health Grants CA38579 and
CA63585.

FIELD OF THE INVENTION

10 This invention relates generally to the fields
of biochemistry, molecular genetics and medicine, and
more specifically to methods for identifying genomic
changes occurring in cancerous cells for the molecular
diagnostics of cancer, and methods of determining the
15 prognostic clinical outcome of subjects with cancer.

BACKGROUND OF THE INVENTION

Genomic instability characterizes neoplastic
transformation and generate tumor cell aneuploidy.
Mutations activate positive regulators of cell growth or
20 survival and inactivate factors with a negative role in
these processes. Losses of heterozygosity (LOH) unmask
recessive mutations in tumor suppressor genes. LOH can
be detected by restriction fragment length polymorphisms
(RFLP) of polymorphic minisatellite loci, and more
25 recently by PCR amplification of highly informative
microsatellite loci. The "allelotype" approach was
critical for the identification and subsequent
characterization of RB and p53 and the emergence of the
tumor suppressor gene era.

But losses of genetic material provide only half of the picture of the distorted genome found in the majority of cancer cells. Chromosomal gains can be diagnostic indicators of the presence of dominant
 5 oncogenes such as c-K-ras. Gains of genetic material may also lead to overexpression of genes contributing to tumor progression in the absence of mutation. Thus, moderate gains (e.g., equivalent to trisomy/tetrasomy) of chromosomal fragments have been long known to be germane
 10 to neoplasia. The detection of such moderate chromosomal changes has been a challenge in cancer research, and perhaps as a consequence, "the pathogenetic significance of such abnormalities is totally unknown" as Mitelman et al., wrote in their recent review.

15 Recent progress in the molecular genetics of cancer has facilitated the detection of allelic abnormalities at the subchromosomal level. Representation differential analysis (RDA) is a powerful technique for the identification and isolation of sequences under and
 20 over-represented in tumor genomes. LOH analysis by RFLP or microallelotyping procedures can be used for the detection of tumor suppressor genes. However, these techniques cannot identify moderate gains of genetic material. Improvements to make PCR quantitative have
 25 been implemented for microallelotyping, but at the expense of losing simplicity. Comparative genomic hybridization (CGH) has allowed the assessment of numerical and structural chromosome aberrations. However, CGH requires special instrumentation and can
 30 only detect alterations of relatively large chromosomal regions.

DNA fingerprinting of polymorphic minisatellites has been used to study anonymous somatic

mutations during tumorigenesis, either by one or two dimensional gel electrophoresis. However, these techniques utilize Southern blot hybridization of genomic DNA and the subsequent isolation and characterization of the altered sequences is difficult.

The Arbitrarily Primed Polymerase Chain Reaction or AP-PCR, as described in U.S. Patent No. 5,487,985, is a PCR based DNA fingerprinting technique using single primers of arbitrarily chosen sequence and several initial cycles of low stringency. Primer annealing at multiple sites generates many PCR products that represent a DNA fingerprint specific for each primer-DNA template combination. Comparison of the AP-PCR fingerprints from matched tumor and normal tissues identifies somatic mutations. AP-PCR DNA fingerprinting was instrumental for the identification of the microsatellite mutator phenotype pathway for cancer. The detection of recurrent fingerprint band shifts revealed the tumor-specific accumulation of hundreds of thousands of somatic clonal mutations. This genome-wide instability in repetitive sequences underlies a mutator phenotype pathway for some sporadic and hereditary gastrointestinal cancers.

The quantitative nature of AP-PCR fingerprinting also allows the detection of allelic losses and gains in tumor cells by the reduction or increase in intensity of tumor fingerprint bands, respectively. The chromosomal origins of most fingerprint bands can be assigned simultaneously by AP-PCR of somatic monochromosome cell hybrid panels. These features offer an excellent opportunity to use AP-PCR DNA fingerprinting as an unbiased molecular karyotyping of tumors.

Evidence supporting the role in tumor development of moderate gains of genetic material is well established (Rabinowitz, Z. & Sachs, L., (1970) *Nature (London)* **225**, 136-139; Spira, J., Wiener, F., Ohno, S. & Klein, G. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 6619-6621; and Klein, G. (1981) *Nature (London)* **294**, 313-318). However, these moderate gains in cancer development have remained essentially unexplored. This is due in part to fashionable changes in the prevalent scientific archetypes: first, there was the excitement of the studies on mutant dominant oncogenes, and later on recessive tumor suppressor genes. These temporal fluctuations depends heavily on the technical advances preceding (Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M., Martin, C., Fujimoto, E., Hoff, M., Kumlin, E., et al., (1987) *Science* **234**, 1616-1622; and Wigler, M., Pellicer, a., Silverstein, S. & Axel, R. (1978) *Cell* **14**, 725-731) the trend-setting studies (Vogelstein, B., Fearon, E.R., Kern, S.E., Hamilton, S.R., Preisinger, A.C., Nakamura, Y. & White, R. (1989) *Science* **244**, 207-211; and Shih, C., Shilo, B.Z., Goldfarb, M.P., Dannenberg, A. & Weinberg, R.A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5714-5718). Although several approaches have been applied to study genetic changes in cancer cells, no simple techniques are available for the study of moderate gains of genetic material.

Despite the progress made in developing powerful analytical tools for examining the molecular genetics of cancer, such as, AP-PCR DNA fingerprinting, there remains an ongoing need to develop better methods for determining the prognostic clinical outcome of a subject with cancer, which would provide the health care

provider with a much needed tool in prescribing the most advantageous course of treatment for such a subject with cancer.

The present invention satisfies this ongoing
5 need and provides additional advantageous aspects as well. It has been surprisingly discovered, that applying AP-PCR DNA fingerprinting to study the prevalence of allelic losses and gains at different stages of colorectal tumor progression, a general method of determining the
10 prognostic clinical outcome of a subject with cancer has been invented.

SUMMARY OF THE INVENTION

A method of determining the clinical outcome of a subject with a cancer using a Genomic Damage Fraction
15 comprising, (a) determining the relative change in quantity of nucleic acids between cancerous cells and non-cancerous cells of said subject, (b) determining the Genomic Damage Fraction from the results of step (a), and (c) determining the prognosis of said subject according
20 to said subject's GDF, where a GDF greater than a predetermined GDF is indicative of a first clinical outcome (e.g., a poor prognosis), and a GDF lesser than a predetermined GDF is indicative of a second clinical outcome (e.g., a good prognosis); and a method of
25 identifying certain genomic sequences whose alterations during tumorigenesis of a subject with a cancer have prognostic value for determining the clinical outcome of said subject comprising, (a) determining the molecular profiles of genomic losses and gains ("amplotyping") of
30 tumors at different stages of progression from the same cancer patient, (b) identifying changes (losses and gains) specifically associated to the more advanced

stages of tumor progression (e.g., metastatic stage), and
 (c) determining the prognosis of said subject according
 to said subject's status of these genomic sequences of
 step b, where a change (loss or gain) is indicative of a
 5 first clinical outcome (i.e., poor prognosis), and no
 change (i.e., no loss or gain) is indicative of a second
 clinical outcome (i.e., good prognosis).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. AP-PCR DNA fingerprints of colorectal tumors.

10 Autoradiogram of a denaturing polyacrylamide sequencing
 gel of the AP-PCR fingerprints generated by arbitrary
 primer MCG1 with 100 ng of genomic DNA isolated from
 normal and tumor tissues from colorectal cancer patients
 indicated at the top. The first, second and third
 15 fingerprint lanes of each case corresponds to normal
 tissue (N), primary tumor (P) and liver metastasis (M).
 N, P and M were available from patients # 1-12, and only
 N and M were available for patients # 13-15. Numbers at
 the left indicate the chromosomal origin of the bands
 20 named by letters at the right. Cases 1,3,6,8,10 and 12
 were males. Band F (designated with an asterisk) was a
 composite of at least three sequences from chromosomes 2,
 11, and 22. Some other bands were composite of sequences
 mainly derived from at least two chromosomes, such as
 25 bands C, L and N (Yasuda, J., Navarro, M., Malkhosyan,
 S., Velazquez, A., Seikya, T. & Perucho, M. (1996)
Genomics **34**,1-7). In these cases, no estimation of
 intensity variations were attempted, except for band C
 (see Detailed Description). Some double bands represent
 30 the two strands of the same DNA molecule (such as bands
 B0, B2, G and J). Band S represents a length
 polymorphism that resolves the two alleles by their
 different size in heterozygous cases (cases 4, 5, 9, 10,

13 and 14). The approximate size of some of the cloned fingerprint bands are: D:800bp; E:750bp; F:710bp; J:575; M: 525bp; and Q: 405 bp.

Figure 2. AP-PCR DNA fingerprints of tumor cell lines of characterized karyotypes.

The cell lines and their gender are indicated at the top. Fingerprints were generated using 40 and 60 ng of DNA each cell line using primer MCG1. The band names are at right and the chromosomes of each band at left. Bands G and J, from chromosomes X and 13 are double bands because the two DNA strands are resolved in these denaturing gels. Band I (chromosome 7) is polymorphic in the human population, including length polymorphisms, and there are non-linear fluctuations in the intensity of the amplified PCR product due to sequence changes in the primer annealing regions. However, comparative analysis of tumor and normal tissues from the same individual is informative for gains or losses of these sequences, since the problem of inter-individual variation is eliminated. These cell lines were chosen because of their (pseudo)diploid nature (all but HT29). SW48 exhibits trisomy of 7, there is only one N13 in HuTu80 cells and LS174-T is monosomic for X (American Tissue Cell Type Collection).

Figure 3. Microallelotyping of colorectal tumors. Microsatellite repeats D13S160 and D13S221 (from chromosome 13q) were amplified by PCR from some of the same genomic DNAs which were used in the experiments of Figure 1 (cases indicated at the top). The radioactive PCR products were analyzed in denaturing sequencing gels.

Figure 4. Chromosome 13 regions of gains and losses in metastatic colon cancer.

The graph depicts chromosome 13 with the position of the Rb locus and the three amplotype bands from the MCG1 primer (A₀, E and J) determined by PCR of radiation hybrid panels (Navarro et al, in preparation), and the

5 dinucleotide repeats analyzed with their approximate localization. Triple bars summarize at right our analysis by amplotyping (left bars), microalleotyping (middle bars) and the combined analysis (right bars), for the metastatic tumors shown at the top. The summary of our

10 studies, including other tumors not shown, is represented with a single bar under "Common regions" in the center of the figure. At the left of the figure is the summary of chromosomal changes observed by CGH (Ried, T., Knutzen, R., Steinbeck, R., Blegen, H., Schrock, E., Heselmeyer,

15 K., du Manoir, S. & Auer, G., (1996) *Genes Chromosomes Cancer* **15**, 234-245), where the thick lines represent chromosomal gains.

Figure 5. Molecular karyotype (amplotype) of metastatic colon cancer.

20 Each bar represents the percent (among the 25 analyzed cancer cases) of loss (lower panel) or gain (upper panel) of a chromosomal region detected as a change of the intensity of a corresponding AP-PCR band. For instance, the three bands from chromosome 8 (bands D and O of MCG1

25 primer and band K from BLUE primer) are represented by three bars on chromosome 8. The data is derived from complete analysis of the fingerprints obtained with two primers, MCG1 and BLUE. A partial analysis of two bands from primer F generated information on the imbalance

30 status of chromosomes 17 and 18.

Figure 6. Comparative amplotypes of primary and metastatic colon cancer.

The symbols are as in Figure 4. Percent indicate the average values of gains and losses from the multiple fingerprint bands for each chromosome (when appropriate, see Fig. 4) in the 12 primary (Dukes' D) and 25 metastatic tumors. The P values of the comparison between primary (P) and metastatic (M) tumors were calculated by the Fisher exact test. Only statistically significant values are shown (losses of chromosome 4 and gains of 6) with the exception of chromosome 12 gains (P=0.003). The P values considering only the 12 cases with primary and metastatic tumors from the same patients were P=0.024 for chromosome 4 losses, and P=0.057 and P=0.099 for chromosome 6 and 12 gains, respectively. Therefore, only the losses of chromosome 4 and gains of chromosome 6 are considered to be significantly increased in metastatic versus primary tumors.

Figure 7. Prognostic value of AP-PCR fingerprinting for colon cancer.

The figure shows the survival curves of colorectal cancer patients according to the losses of a fingerprint band from chromosome 4. A panel of 35 colorectal carcinomas with follow-up information after surgical resections with curative intent (Arribas, R., Capella, G., Tortola, S., Masramon, L., Grizzle, W.E., Perucho, M. & Peinado, M.A., (1997) J. Clin. Oncol. 15, 3230-3240) were analyzed by AP-PCR DNA fingerprinting, and the differences in disease free survival time were compared relative to the alterations observed in the fingerprint bands. Kaplan-Meier disease free survival curves are plotted according to the allelic status of band N of the BLUE arbitrary primer fingerprints (Yasuda, J., Navarro, M., Malkhosyan, S., Velazquez, A., Seikya, T. & Perucho, M. (1996) *Genomics* 34,1-7). Losses of this band are linked to

increased risk, independently of Dukes stage (RR: 2.6, 95% CI: 1.0-6.4, $p=0.0427$).

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention,
5 methods are provided for determining the clinical outcome of a subject with a cancer using a Genomic Damage Fraction comprising,
a. determining the relative change in quantity of nucleic acids between cancerous cells and
10 non-cancerous cells of said subject;
b. determining the Genomic Damage Fraction from the results of step (a);
c. determining the prognosis of said subject according to said subject's GDF, where a GDF greater than
15 a predetermined GDF is indicative of a first clinical outcome (e.g., a poor prognosis), and a GDF lesser than a predetermined GDF is indicative of a second clinical outcome (e.g., a good prognosis).

For example, one aspect of this embodiment is
20 where the relative change in quantity of nucleic acids is determined using AP-PCR DNA fingerprinting. Other aspects of this embodiment is where the first clinical outcome is increased risk; where said second clinical outcome is decreased risk; where the relative change in
25 nucleic acids is determined by the number of quantitative and/or qualitative changes in the DNA fingerprint bands present in the cancerous cells as compared with the normal cells; where the relative change in nucleic acids is determined by the number of quantitative changes in
30 the DNA fingerprint bands; where the relative change in nucleic acids is determined by the number of qualitative changes in the DNA fingerprint bands; where the relative

change in nucleic acids is determined by the number of quantitative and qualitative changes in the DNA fingerprint bands; where the relative change in nucleic acids is a gain or loss in quantity in nucleic acids; 5 where the relative change in nucleic acids is a gain in quantity in nucleic acids; where the relative change in nucleic acids is a loss in quantity in nucleic acids; or where the subject with cancer has colorectal cancer.

Another embodiment of the present invention, is 10 a method of determining the clinical outcome of a subject with a cancer comprising,

- a. generating the AP-PCR DNA fingerprint of non-cancerous cells from said subject;
 - b. generating the AP-PCR DNA fingerprint of 15 primary cancer cells from said subject;
 - c. generating the AP-PCR DNA fingerprint of metastatic cancer cells from said subject; and
 - d. identifying chromosomal regions from AP-PCR DNA fingerprint data of steps (a), (b) and (c)
- 20 wherein the occurrence of gains or losses of nucleic acids in certain chromosomal regions is prognostic of the clinical outcome for said subject.

For example, one aspect of this embodiment is where the gain or loss of nucleic acids is significantly 25 different in metastatic cancer cells as compared to primary cancer cells; where the chromosomal region is determined by a band of chromosome 4 obtained using the BLUE primer (SEQ ID No: 1); or where the band is band N from the DNA fingerprint generated with the BLUE primer.

30 Still another embodiment of the present invention is a method of determining the clinical outcome of a subject with a cancer comprising,

a. generating the AP-PCR DNA fingerprint of non-cancerous cells from said subject;

b. generating the AP-PCR DNA fingerprint of primary cancer cells from said subject;

5 c. identifying chromosomal regions from AP-PCR DNA fingerprint data of steps (a) and (b), where gains or losses of nucleic acids occur; and

d. comparing said AP-PCR DNA fingerprints of chromosomes 1, 4, 6, 8, 9, and 13 from step a and step b
10 wherein presence of gain or loss of nucleic acids in certain chromosomal regions is prognostic of the clinical outcome for said subject.

For example, one aspect of this embodiment is where the chromosomal region that is determined by band N
15 of chromosome 4 from the Blue primer fingerprint is prognostic of the clinical outcome for said subject.

Yet another embodiment of the present invention is a method of predicting a clinical outcome of a subject with cancer using an amplotype from said subject
20 comprising,

a. locating chromosomal regions that have gained and lost nucleic acids using AP-PCR DNA fingerprinting;

b. identifying said chromosomal regions that
25 have lost nucleic acids; and

c. identifying said chromosomal regions that have gained nucleic acids;
wherein the combination of gains and losses according to chromosomal regions are prognostic of the clinical
30 outcome for subject with cancer.

For example, one aspect of this embodiment is where the results of step (b) and step (c) are displayed

where said gains and losses of nucleic acids are listed according to the chromosomal regions where they occur, wherein the combination of gains and losses according to chromosomal regions are prognostic of the clinical
 5 outcome for subject with cancer.

Definitions

As used herein, the term "Blue Primer" refers to the nucleic acid sequence, 5' CCG AAT TCG CAA AGC TCT GA 3' (SEQ ID NO: 1).

10 As used herein, the term "Genomic Damage Fraction", or "GDF" refers to a measure of the change in quantity of nucleic acids between non-normal cells (e.g., cancerous cells) and normal cells in an individual. A predetermined GDF value is established by measuring the
 15 GDFs of a group of individuals with a cancer and correlating this information with actual clinical outcome for the individuals.

As used herein, the term "GDF_G" refers to a measure of the gain in quantity of nucleic acids between
 20 non-normal cells (e.g., cancerous cells) and normal cells in an individual.

As used herein, the term "GDF_L" refers to a measure of the loss in quantity of nucleic acids between non-normal cells (e.g., cancerous cells) and normal cells
 25 in an individual.

As used herein, the term "AP-PCR DNA fingerprinting" refers to a technique for the rapid generating of a set of discrete DNA amplification

products characteristic of a genome as a fingerprint as described in U.S. Patent No. 5,487,985 "McClelland '985"

As used herein, the term "amplotype" or "amplotyping" refers to the process of generating the AP-PCR DNA fingerprint of cancerous cells (e.g., primary cancer cells or metastatic cancer cells) and non-cancerous cells of a subject with cancer, and assembling the AP-PCR DNA fingerprint information according to gain and loss of nucleic acid material per chromosome where certain combinations of chromosomes gaining or losing nucleic acids is prognostic of the clinical outcome of the subject.

As used herein, the term "determining the clinical outcome of a subject with cancer" refers to whether the subject will have increased risk from the recurrence of the cancer, e.g., poor prognosis, such as, an increased rate of progress of a cancer in a subject, and/or the increased likelihood the cancer will become metastatic. Conversely, whether the subject will have decreased risk from recurrence of cancer, e.g., good prognosis, such as, a decreased rate of progress of a cancer in a subject, and/or a decreased likelihood the cancer will become metastatic.

Genomic instability characterizes the aneuploid cancer cell. Losses of genetic material are critical in cancer by exposing recessive mutations in tumor suppressor genes. Gains of genetic material may also lead to overexpression of genes contributing to tumor progression either in the presence or absence of mutation. However, the detection of moderate gains (such as tri-tetraploidy) has been a challenge in cancer research. Unbiased DNA fingerprinting by the Arbitrarily

Primed PCR (AP-PCR) allows the detection moderate gains (in addition to losses) of DNA sequences of known chromosomal localization. Using AP-PCR DNA fingerprinting in this manner, a molecular karyotype of metastatic colon cancer is generated. This amplotype shows that sequences from several chromosomes undergo both losses (1, 4, 9, 14 and 18) and gains (6, 7, 12 and 20) in over half of the tumors. Moreover, gains of sequences from chromosomes 8 and 13 occurred in most tumors, indicating the existence in these chromosomes of positive regulators of cell growth or survival which are under strong positive selection during tumor progression. The over representation of these chromosomal regions is a critical step for metastatic colorectal cancer.

Comparative amplotype analysis from primary and metastatic tumors shows the existence in chromosome 4 of gene(s) whose loss is specifically selected in cells that reach the metastatic stage.

Unbiased DNA fingerprinting of colorectal cancer.

AP-PCR DNA fingerprinting is applied to the analysis of chromosomal numerical changes in human colorectal cancer. Two arbitrary primers, MCG1 and BLUE, were selected based on their fingerprints quality (low background) and quantity (more than 25 bands). The chromosomal origin for most of the fingerprint bands was previously determined (Yasuda, J., Navarro, M., Malkhosyan, S., Velazquez, A., Seikya, T. & Perucho, M. (1996) *Genomics* **34**,1-7). Each autosome was represented by at least one fingerprint band, except chromosomes 18, 19 and 21. Therefore, estimation of band losses and gains of the fingerprints generated by these two primers allowed to establish a molecular karyotype of colorectal cancer. This molecular karyotype is called an

"amplotype", to distinguish it from the conventional "allelotype", whereby only LOH, and by inference allelic losses, can be determined. The metastatic tumors are analyzed to determine the amplotype with the presumed
 5 highest number of chromosomal changes.

Figure 1 shows the AP-PCR fingerprints generated by the arbitrary primer MCG1. Differences in band intensity are frequent in the DNA fingerprints from normal versus tumor tissues. Some of these differences
 10 are due to variation in the overall levels of amplification between DNAs (compare the backgrounds of cases 10, 12 and 14) and are not considered significant (for instance the increased intensity of band A in the metastasis of case 14). The intensity changes of other
 15 bands are on the other hand reproducible (see Examples for the criteria followed for scoring gains and losses). Because of the complexity of the figure, we describe only some of the representative bands: D and O, derived from chromosome 8, and E and J, derived from chromosome 13.
 20 These bands showed consistent increases in tumor tissue DNAs, in contrast with other bands exhibiting sporadic intensity changes, such as the increased intensity of band A in cases 2 and 7 in both primary (P) and metastatic (M) tumors, and case 8 (only M). Examples of
 25 increases in bands E and J: cases 1 (only band J, in both P and M); 2, 7 and 8 (both bands E and J, in both P and M); 3 (only band E, in both P and M). Examples of increases in bands D and O: cases 4, 5, and 12 (both bands D and O, in both P and M); 8, 9, 10 and 11 (band O, in both P and M; band D, in P); and cases 13 and 14 (both
 30 bands D and O).

Application of the Genomic Damage Fraction

The "Genomic Damage Fraction", or "GDF" is a measure of the change in quantity of nucleic acids between non-normal cells (e.g., cancerous cells) and normal cells in an individual. Typically, the GDF is used to compare normal cells with tumor cells, and also, normal cells with primary cancer cells and metastatic cancer cells. For example, the GDF can be derived using AP-PCR DNA fingerprinting techniques. The AP-PCR technique is used to generate DNA fingerprints of normal and non-normal cells. The DNA fingerprints are presented in the form of bands of nucleic acid materials representative of certain chromosomal regions for the subject as resolved by electrophoresis gel, e.g., as depicted in Figure 1. The DNA fingerprint of normal cells is by definition the baseline standard. Generally, the DNA fingerprint of the cancerous cells will be different from that of non-cancerous cells, where the cancerous cells will have additional and/or stronger bands (i.e., gain of nucleic acid material) as compared with the non-cancerous cells, or missing and/or weaker bands (i.e., loss of nucleic acid material) as compared with the non-cancerous cells. The total number of deviations, i.e., number of occurrences of additional and/or stronger bands and of missing and/or weaker bands, is divided by the total number of bands present in the non-cancerous cells resulting in the GDF.

A measure of the gain in nucleic acids in the non-normal cells can be generated from the number of bands corresponding to gained nucleic acid is divided by the total number of bands present in the non-cancerous cells resulting in the GDF_G .

A measure of the loss in nucleic acids in the non-normal cells can be generated from the number of bands corresponding to lost nucleic acid is divided by the total number of bands present in the non-cancerous
 5 cells resulting in the GDF_L .

The intensity of the bands vary with the density of nucleic acids aggregating at a given position on an electrophoresis gel. A band with stronger intensity in the fingerprint from tumor tissue DNA
 10 indicates the existence of more DNA fragments in the fingerprint gel and by extrapolation, of more DNA molecules in the donor tumor tissue cells relative to the normal tissue cells, for example, by gains of nucleic acids in the region of the tumor cell genomes
 15 corresponding to the sequences of the particular fingerprint band. Conversely, a fainter band in the tumor tissue fingerprint represents the loss of the corresponding genomic sequences. A complete loss of a particular sequence, for example, a homozygous deletion,
 20 is reflected by the absence of the band. While a partial loss of the sequence, for example, a heterozygous deletion - loss of one of the two alleles of a particular sequence, is reflected by a fainter band. Similarly, a gain of a few copies of a particular allelic sequence
 25 will be reflected in a band of moderately stronger intensity in the tumor fingerprint, for example, double or triple intensity. A gain of many copies of a particular sequence will be reflected by a more drastic increase in intensity. When this occurs with a genomic
 30 sequence that generates a very faint band in the normal tissue fingerprint, the amplification of the sequence in the tumor cell genome may appear as a new band in the tumor fingerprint. Both stronger and weaker fingerprint bands represent therefore relative quantitative changes

in nucleic acids content between tumor and normal tissues.

Qualitative changes in APPCR fingerprints are on the other hand changes that are due to structural alterations in the genome of the tumor cell. Thus, a chromosomal rearrangement such as a translocation, or a deletion or a insertion of a particular segment of the genome may result in a new band in the fingerprint. Examples of such qualitative alterations are the deletion mutations that are very common in tumors of the microsatellite mutator phenotype (Ionov, Y., Peinado, M.A., Malkhosyan, S., Shibata, D. & Perucho, M. (1993) *Nature* (London) 363, 558-561). These ubiquitous deletion mutations in simple repetitive sequences or microsatellites are reflected by a change in the mobility of some fingerprint bands (i.e., a new band appears in the tumor fingerprint). The GDF may or may not incorporate these qualitative changes.

Not all bands are of the same intensity in each fingerprint, some bands are more intense than others. Therefore, the actual number of bands discernable and hence recorded will vary according to the degree of sensitivity for detection. Although this is potentially a source of variability in the measure from practitioner to practitioner, as long as the degree of detection is applied consistently by each practitioner to designate whether a band is present or absent, the ultimate value of GDF will be consistent, because any differences between practitioners is negated by virtue of the GDF being a ratio of the change in number bands and total number of bands.

It should also be noted that the GDF is normalized for differences within a given individual and therefore can be used as a measure between different individuals, or populations of individuals. As such the GDF can be used as a quantitative measure of the change in quantity of nucleic acids between normal and non-normal cells in an individual. GDF may also be used as a qualitative measure between a first individual and a second individual, or first individual and a population of individuals. For this reason, GDF is a useful tool for epidemiological studies of diseases that are associated with changes in the quantity of nucleic acids in individuals.

GDF provides a quantitative measure of the genomic damage evidenced in cancer cells against non-cancer cells in a subject. The GDF can also provide a qualitative measure of a subject's survivability when the subject's GDF is compared with an established GDF value, e.g., where a subject with a GDF higher than an established value is indicative of greater risk for the recurrence of the disease, and a GDF lower than the established value is indicative of lesser risk for the recurrence of the disease.

Allelic losses and gains in colorectal cancer.

Southern blot hybridization experiments with cloned bands E, J and D showed that tumor-specific increased intensity of these fingerprint bands was due to the higher copy number of target sequences, and not an artifact of the *in vitro* amplification (Peinado, M.A., Malkhosyan, S., Velazquez, A & Perucho, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10065-10069 data not shown). The quantitative nature of the amplification levels is

also illustrated by the concordance of the relative intensities of band G from chromosome X with the gender of the cancer patients (Figure 1). The ability of AP-PCR DNA fingerprinting to detect moderate changes of chromosome copy number is also shown in the fingerprints of tumor cell lines of known karyotype (Figure 2). In addition to the correspondence of chromosome X band intensity with the gender of the donors, loss of an autosome is reflected by the decreased intensity of band J from chromosome 13 in the HuTu80 cell line, monosomic for this chromosome. The intensity variation of band gains (see Examples) ranged from 2.276 ± 1.27 in tumor DNA in the case with higher level of gains (chromosome 13 band E), which we estimate represents 3 to 7 copies.

Ambiguity of microallelotyping for determining the nature of allelic imbalances.

We carried out PCR amplification of two chromosome 13 microsatellite loci (Figure 3) to determine the relationship between the chromosomal imbalances detected by fingerprinting and by microsatellite analysis, commonly used for the estimation of LOH in tumors (microallelotyping). The results show that microallelotyping may be misleading to interpret the allelic composition of the loci analyzed. Thus, in tumors 7, 8 and 16, the results can be interpreted as indicative of LOH at the D13S221 locus and in tumors 8 and 16 of the D13S160 locus. However, as shown by the fingerprints (Figure 1), in concert with Southern blot hybridization (data not shown), the consistent change in these tumors was the gain but not the loss of chromosome 13 sequences. These results show that microallelotyping only detects allelic imbalances because an apparent loss

of one allele in tumor compared to normal tissue can be due to the gains of the other allele.

While the use of microallelotyping alone is insufficient to determine gains, in combination with
 5 amplotyping provides additional information on the chromosomal alterations undergone in the aneuploid cancer cell. For instance, while in the telomeric chromosome 13 region both alleles appear to be gained, in the more centromeric 13q14-q21 region, gains of one allele are
 10 accompanied by the losses of the other (Figure 4).

Amplotype of colorectal cancer.

The global results of these analyses are represented in Figure 5. The frequency of losses and gains was similar. An average of near 25% of all
 15 chromosomal regions analyzed exhibited losses or gains per tumor and their combined values reached near 50%. The average frequency of chromosomal losses is slightly higher than the fractional allelic loss (FAL) determined by allelotyping (Vogelstein, B., Fearson, E.R., Kern, S.E., Hamilton, S.R., Preisinger, A.C., Kakamura, Y. &
 20 White, R. (1989) *Science* **244**, 217-221). This may be explained because of the more advanced stage of progression of our tumors. On the other hand, the amplotype approach is not as sensitive to detect allelic
 25 losses as the allelotype procedure because loss of one allele and reduplication of the other would score positive by allelotyping but negative by amplotyping. This situation is revealed by the combination of fingerprinting, Southern blot and microallelotyping. For
 30 instance, case 7, shows that the intensity of the D band in the fingerprint is not decreased (Figure 1). However, allelotyping of these bands showed the loss of one allele

of the chromosome 8 D band in both primary and metastatic tumor (data not shown), indicating that the loss of one allele was accompanied by the reduplication of the other. Similar conclusion can be reached for the length polymorphic band S from chromosome 12 in both tumor tissues of case 9 (Figure 1). The gain of the long allele compensates the loss of the short.

The aneuploidy of metastatic colon cancer (Figure 5) shows that losses of sequences from chromosomes 1, 4, 9, 14 and 18 occurred in about 50% of the tumors. Over 50% of tumors also exhibited gains of bands from chromosomes 6 and 20 and over 75% of tumors exhibited gains in multiple bands from chromosomes 8 and 13.

15 **Chromosomal imbalances in primary and metastatic colon cancer.**

The availability of 12 cases with both primary and metastatic tumors allowed to investigate whether any of the chromosomal changes were metastasis-specific. Most consistent gains or losses were not significantly associated with metastatic cancer. For instance, neither the gains of chromosome 8 or 13 were events specific for the metastatic process, but to precede it. However, chromosome 4 losses and chromosome 6 gains were significantly associated to the metastatic stage (Figure 6).

AP-PCR DNA fingerprinting can be used to detect genetic alterations during tumorigenesis. This approach presents several advantages compared with other techniques. One PCR reaction allows to quantitatively compare normal and tumor tissues at multiple sites of the

genome and also permits the single-step cloning of DNA fragments representing altered genomic sites (Peinado, M.A., Malkhosyan, S., Velazquez, A & Perucho, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10065-10069). It also

5 gives an overall picture of the extent of genetic damage in tumor cells which may have prognostic value for cancer (Arribas, R., Capella, G., Tortola, S., Masramon, L., Grizzle, W.E., Perucho, M. & Peinado, M.A. (1997) *J. Clin. Oncol.* **15**, 3230-3240). The technique also

10 permits the simultaneous identification in many tumor samples of moderate allelic losses and gains. The genomic localization of these loci can be previously determined at the chromosomal or subchromosomal levels by the use of somatic human-rodent monochromosome or

15 radiation hybrids (Peinado, M.A., Malkhosyan, S., Velazquez, A & Perucho, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10065-10069; and Yasuda, J., Navarro, M., Malkhosyan, S., Velazquez, A., Seikya, T. & Perucho, M. (1996) *Genomics* **334**, 1-7 and work in progress).

20 Consequently, this approach represents a molecular tool of high resolution for cancer cytogenetics.

Among the novel findings in this study is the existence of potential new tumor suppressor genes for colorectal cancer at chromosomes 1 and 9 and for the

25 metastatic stage at chromosome 4. There are no reports of recurrent chromosome 4 losses nor of metastasis-specific cancer genes in colorectal cancer. This underscores the power of unbiased DNA fingerprinting to identify new genomic regions containing potential tumor suppressor

30 genes. In addition, losses of one of the bands from chromosome 4 have value as prognostic indicator for increased risk to colorectal cancer. Independent AP-PCR analysis of a collection of colorectal carcinomas for

which follow-up information was available (Arribas, R., Capella, G., Tortola, S., Masramon, L., Grizzle, W.E., Perucho, M. & Peinado, M.A. (1997) *J. Clin. Oncol.* **15**, 3230-3240) revealed that among the fingerprint band
 5 displaying recurrent alterations, somatic loss of the Blue primer band N from chromosome 4 (Yasuda, J., Navarro, M., Malkhosyan, S., Velazquez, A., Seikya, T. & Perucho, M. (1996) *Genomics* **34**, 1-7) was a prognostic indicator of poor survival (Figure 7). Therefore, the
 10 association of the losses of these sequences with the metastatic stage may be explained by assuming that the tumors that undergo the losses of these sequences have an enhanced ability to metastasize.

Thus, another embodiment of the present
 15 invention is a method of identifying genomic regions relevant for cancer. The method is exemplified by the experiment described in Figure 1. It is performed in a single denaturing gel electrophoresis by PCR amplification with a single arbitrary primer of the DNA
 20 from normal, primary and metastatic tissues of a panel of cancer patients, yielding relevant information for cancer diagnosis and prognosis, for example as exemplified in the amplotype of Figure 5. In combination with follow-up information of the corresponding cancer patients, (e.g.,
 25 continued monitoring of the physical progression or regression of the tumor, the recurrence of the tumor after either surgical intervention or radiation therapy, development of other related or unrelated tumors in the patient, and the like) the task of finding prognostic
 30 markers for cancer is facilitated by focusing on only the minority of sequences that are likely to be useful prognostic markers (i.e., those fingerprint bands tightly linked to metastatic-specific cancer genes). While the procedure does not identify the actual responsible cancer

gene (i.e., the metastatic gene), it identifies a genomic region that is closely linked to the cancer gene, thus facilitating the subsequent task of gene hunting.

While the specific example of the invention is
5 a band undergoing frequent losses in metastatic but not primary cancers, other situations can be envisioned where the prognostic marker will be the gain of a specific sequence more frequently in metastatic than in primary cancers.

10 In addition, once identified the relevant prognostic marker, and its rapid isolation from the fingerprint gels provides a further simplification of the experimental approach of generating useful cancer prognostic markers. For example, mapping of the band to
15 the chromosomal region at 4p16 allows one of ordinary skill in the art to immediately identify other close polymorphic markers in the same chromosomal region, such as, D4S339 and D4S524 (The Genome Database (<http://gdbwww.gdb.org/>)). This then facilitates the task
20 of the screening of tumors for losses of this chromosomal region by the standard microallelotyping approach. Detection of losses of heterozygosity (LOH) in any of these adjacent dinucleotide microsatellite markers may be also useful for cancer prognosis, and more amenable to
25 routine testing in clinical settings.

The other novel finding of our study is that moderate gains of chromosome sequences are equally prevalent as the losses. While determination of losses is "digital" (yes or no), the extent of chromosomal
30 sequence gains represented by the fingerprint bands is "analogical", since in principle there is no upper limit. However, we estimate that these gains represented no more

than 5-7 copies in any of the cases studied with the arbitrary primers used in this work. Detection of typical amplicons (undergoing more than 10 fold amplification) by AP-PCR DNA fingerprinting can be achieved by using more primers (Okazaki, T., Takita J., Kohno, T., Handa, H. & Yokota, J. (1996) *Hum. Genet.* **98**, 253-258). The lower limit of detection of chromosomal gains appears to be 3, but due to sensitivity limitations of the method, triploidy cannot formally be distinguish from tetraploidy. However, in contrast with the "digital" losses, the functional difference of an "analogical" gain of three versus four copies seems not so critical.

We also found that gains of some chromosomes occur with a frequency significantly higher than previously reported by cytogenetic and molecular cytogenetic (CGH) approaches. This can be explained because our approach to detect gains is independent of the location of the gained sequences either in a chromosome recognizable cytogenetically, or within a chromosomal region of a minimum size to be detectable by CGH. The high frequency of moderate gains of chromosomes 6, 8 and 13, illustrates the importance for tumor progression of chromosomal imbalances leading to moderate over representation of gene products.

It has been shown that DNA fingerprinting by AP-PCR fulfills the requirements for a technique, that because of its simplicity and sensitivity, it facilitates the estimation of the prevalence of moderate gains of genetic material in tumors. These gains imply the existence of a gene or a set of genes in the corresponding chromosomes whose moderate gains are selected during tumor progression, probably because their

products confer a selective advantage for growth or survival to the tumor cells. This hypothesis is based on the principle that if the same somatic mutation occurs independently and in a consistent manner, it is probably
 5 relevant for tumor development (Baker, S.J., Fearon, E.R., Nigro, J.M., Hamilton, S.R., Preisinger, A.C., Jessup, J.M., van Tuinen, P., Ledbetter, D.H., Barker, D.F., Nakamura, Y., et al. (1989) *Science* **244**, 217-221; and Perucho, M., Goldfarb, M., Shimizu, K., Lama, C.,
 10 Fogh, J. & Wigler, M. (1981) *Cell* **27** 467-476).

Gains of chromosome 8q have been described in colorectal cancer by cytogenetics and molecular cytogenetics. The frequency of such gains, often due to trisomy, range from 20-50% by cytogenetics: 9 of 18
 15 (Muleris, M., Salmon, R.J. & Dutrillaux, B. (1990) *Cancer Genet. Cytogenet.* **46** 143-156); 36 of 116 (Bardi, G., Sukhikh, T., Pandis, N., Fenger, C., Kronborg, O. & Heim, S. (1995) *Genes Chromosomes Cancer* **12**, 97-109); or by CGH: 8 of 16 (Ried, T., Knutzen, R., Steinbeck, R.,
 20 Blegen, H., Schrock, E., Heselmeyer, K., du Manoir, S. & Auer, G., (1996) *Genes Chromosomes Cancer* **15**, 234-245). Using AP-PCR fingerprinting there is a frequency of gains of 76% by scoring individual fingerprint bands (Figure 5). These three fingerprint bands have been localized to
 25 8q subchromosomal regions (at a resolution of a few megabases) by the use of radiation hybrid panels (manuscript in preparation). The data does not excludes *c-Myc* as the most obvious candidate for the 8q gene selected for during tumor progression. There are
 30 numerous reports on *c-Myc* amplification in colon cancer (Marcu, K.B., Bossone, S.A. & Patel, A.J. (1992) *Annu. Rev. Biochem.* **61**, 809-860 and references wherein). However, none of these reports reached a frequency as

high as that obtained by amplotyping, probably due to the difficulties in determining moderate levels (equivalent to trisomy/tetrasomy) of gains by molecular hybridization approaches. Therefore, the role of *c-Myc* activation in colorectal cancer might be underestimated. Nevertheless, *c-Myc* may not be the only functional locus in the 8q gains, and the chromosomal region undergoing over representation may contain additional relevant genes for tumor progression (Okazaki, T., Takita, J., Kohno, T., Handa, H. & Yokota, J. (1996) *Hum. Genet.* **98**, 253-258).

Similar analysis of the chromosome 13 gains yields a more complex pattern, because the three fingerprint bands A₀, E and J are concomitantly over represented in many cases (Figure 4). Work in progress is aimed to localize more precisely the common region(s) of gain of this chromosome, which have been also previously implicated in colon cancer by cytogenetic: 10 of 18 (Muleris, M., Salmon, R.J. & Dutrillaux, B. (1990) *Cancer Genet. Cytogenet.* **46** 143-156); 37 of 116 (Bardi, G., Sukhikh, T., Pandis, N., Fenger, C., Kronborg, O. & Heim, S. (1995) *Genes Chromosomes Cancer* **12**, 97-109); RFLP/LOH: 10 of 31 (Lothe, R.A., Fosslie, T., Danielsen, H.E., Stenwig, A.E., Nesland, J.M., Gallie, B. & Borresen, A.L., (1992) *J. Natl. Cancer Inst.* **84**, 1100-1108); and CGH: 8 of 16 (Ried, T., Knutzen, R., Steinbeck, R., Blegen, H., Schrock, E., Heselmeyer, K., du Manoir, S. & Auer, G., (1996) *Genes Chromosomes Cancer* **15**, 234-245) and 5 of 12 (Schlegel, J., Stumm, G., Scherthan, H., Bocker, T., Zirngibl, H., Ruschhoff, J. & Hofstadter, F. (1995) *Cancer Res* **55**, 6002-6005) analyses, but with lower frequency. Because of this high incidence, the over representation of 13q loci is a

nearly obligatory step for the late stages of colorectal cancer.

It is also shown that microallelotyping by PCR amplification of dinucleotide repeats exhibits an
 5 intrinsic ambiguity on the determination of losses or gains of the polymorphic alleles, which may be misleading to identify the relevant alteration (Figure 3). Thus, microallelotyping may be only informative to determine allelic imbalances (Ah-See, K.W., Cooke, T.G., Pickford,
 10 I.R., Soutar, D. & Balmain, A. (1994) *Cancer Res* **54**, 1617-1621) but not LOH (Nawroz, H., van der Riet, P., Hruban, R.H., Koch, W., Ruppert, J.M. & Sidransky, D., (1994) *Cancer Res.* **54** 1152-1155) which are not equivalent. However, in concert with DNA fingerprinting,
 15 microallelotyping may add critical information on the mechanisms underlying these chromosomal alterations. Our results indicate that at least in some cases, the relevant alteration is the quantitative increase in genetic material, because there appear to be a selection
 20 for gains of either of the two alleles. For instance, in some cases, one of the chromosome 13q alleles is over represented in the primary tumor, but the other in the metastasis (Figure 4). In these situations, the gained chromosome probably harbors wild type allele(s). On the
 25 other hand, gains of some sequences may be also accompanied by LOH (for instance, see Figure 1 case 9, band S; Figure 4 and (Achille, A., Biasi, M.O., Zamboni, G., Bogina, G., Magalini, A.R., Pederzoli, P., Perucho, M. & Scarpa, A., (1996) *Cancer Res.* **56**, 3808-3813),
 30 suggesting the co-existence of positive and negative regulators of cell growth or survival closely located in the same chromosomal region. These findings may be disclosing a protective mechanism for cancer development, because it would hinder the mutational

unmasking of the oncogenic potential latent in these chromosomal regions.

The following examples are given to enable those of ordinary skill in the art to more clearly understand and to practice the present invention. The 5 examples should not be considered as limiting the scope of the invention, but merely as be illustrative and representative thereof.

10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
200
205
210
215
220
225
230
235
240
245
250
255
260
265
270
275
280
285
290
295
300
305
310
315
320
325
330
335
340
345
350
355
360
365
370
375
380
385
390
395
400
405
410
415
420
425
430
435
440
445
450
455
460
465
470
475
480
485
490
495
500
505
510
515
520
525
530
535
540
545
550
555
560
565
570
575
580
585
590
595
600
605
610
615
620
625
630
635
640
645
650
655
660
665
670
675
680
685
690
695
700
705
710
715
720
725
730
735
740
745
750
755
760
765
770
775
780
785
790
795
800
805
810
815
820
825
830
835
840
845
850
855
860
865
870
875
880
885
890
895
900
905
910
915
920
925
930
935
940
945
950
955
960
965
970
975
980
985
990
995

EXAMPLES**EXAMPLE 1**

Method of Identifying Genes Associated
With Colorectal Cancer

5 Tumor samples.

Primary colorectal tumors their normal tissue counterparts included 12 primary Dukes' D colon carcinomas and 25 colon cancer liver metastases (12 of these samples were derived from the same 12 primary carcinoma patients). Most of these tumors were obtained from the National Cancer Center at Tokyo. In agreement with the low incidence of metastasis in colon cancer of the microsatellite mutator phenotype (Ionov, Y., Peinado, M.a., Malkhosyan, S., Shibata, D. & Perucho, M. (1993) *Nature (London)* **363**, 558-561), none of the tumors studied exhibited enhanced microsatellite instability. Some metastatic colorectal carcinomas and a panel of 80 tumors at earlier stages of tumor progression with follow-up data (Arribas, R., Capella, G., Tortola, S., Masramon, L., Grizzle, W.E., Perucho, M. & Peinado, M.A. (1997) *J. Clin. Oncol.* **15** 3230-3240) were obtained from the Human Tissue Cooperative Network (University of Alabama, Birmingham).

AP-PCR DNA fingerprinting.

25 Genomic DNA was prepared from tumor and normal tissues as described (Peinado, M.A. Malkhosyan, S., Velazquez, A., & Perucho, M., (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10065-10069; and Arribas, R., Capella, G., Tortola, S., Masramon, L., Grizzle, W.E., Perucho, M. &

Peinado, M.A. (1997) *J. Clin. Oncol.* **15**, 3230-3240.
 DNA (50-100 ng) was subjected to AP-PCR amplification in
 25 ml of reaction mix: 1 unit of Taq DNA polymerase
 (Perkin-Elmer-Cetus), 10 mM of Tris-HCl (pH 8.3), 50 mM
 5 of KCl, 4.5 mM of Mg Cl₂, 0.1% gelatin, and 1 mM of
 primer. The AP-PCR conditions were as previously
 described in (Peinado, M.A. Malkhosyan, S., Velazquez,
 A., & Perucho, M., (1992) *Proc. Natl. Acad. Sci. USA*
89, 10065-10069) with 25 high stringency cycles. The
 10 number of cycles is an important parameter to maintain
 linearity of amplification and is determined empirically
 for each primer. The PCR products were electrophoresed
 in a 5.5% polyacrylamide gel (Peinado, M.A. Malkhosyan,
 S., Velazquez, A., & Perucho, M., (1992) *Proc. Natl.*
 15 *Acad. Sci. USA* **89**, 10065-10069) at 55 W for 5 to 6
 hours. The MCG1 and BLUE arbitrary primer's sequences
 have been described in (Yasuda, J., Navarro, M.,
 Malkhosyan, S., Velazquez, A., Seikya, T. & Perucho, M.
 (1996) *Genomics* **34**, 1-7). The sequence of the F primer
 20 is 5' ATT CAA GAC TGC CTT TCC TA 3'.

Chromosomal assignment of AP-PCR fingerprint bands.

Chromosome assignment was determined by PCR of
 monochromosome human- rodent cell hybrids NIGMS panels 1
 and 2 (Coriell Cell Research) using specific primer sets
 25 previously designed based on the sequence of the cloned
 fragments. Bands were extracted with 100 ml of distilled
 water and reamplified with the same arbitrary primer and
 cloned using the PCR script system (Stratagene) and the
 TA cloning system (Invitrogen) following the
 30 manufacturers' instructions. To confirm the authenticity
 of the cloned bands, they were used as probes in Southern
 blots of AP-PCR gels (Perucho, M., Welsh, J., Peinado,

M.A., Ionov, Y. & McClelland, M. (1995) *Methods Enzymol.* **254**, 275-290). Correct clones were selected by comparison of the fingerprint and blotting patterns. The chromosomal origin of other fingerprint bands was
 5 determined by the SHARP method (Yasuda, J., Navarro, M., Malkhosyan, S., Velazquez, A., Seikya, T. & Perucho, M. (1996) *Genomics* **34**, 1-7). Genomic Southern blot analysis of cloned AP-PCR bands was done as described (Peinado, M.A. Malkhosyan, S., Velazquez, A., & Perucho, M., (1992)
 10 *Proc. Natl. Acad. Sci. USA* **89**, 10065-10069).

Densitometrical analysis.

The dried gels were exposed to X-ray film at room temperature without image intensifier or at -70°C with image intensifier at different time exposures.
 15 Autoradiograms were scanned with ImageMaster Desk Top Scanner (Pharmacia LKB). The scanned image was analyzed with ImageMaster software (Pharmacia) using an IBM-PC PS/V personal computer. The backgrounds of the X-ray film and of the individual lanes were subtracted using
 20 the Rolling-Disk background subtraction method following the instructions by the manufacturer. Film calibration was made by serial dilution of ³²P-labeled oligonucleotides, and the radioactivity was standardized by Cerenkov counting using LS3801 liquid scintillation
 25 system (Beckman), with the same exposure conditions than the AP-PCR fingerprinting gels.

Determination of the status of gains and losses.

Scoring quantitative changes between normal and tumor tissue fingerprint bands was made by

densitometrical analysis and by visual inspection. To establish the criteria for gain and loss in the densitometrical analysis, the data of the fingerprints of normal samples were calibrated. The mean standard
5 deviations of non-polymorphic bands were estimated to be around 10%. In other words, the range of fluctuations in band intensity due to experimental variation usually was between 0.9 to 1.1. Considering the contamination of tumor tissue by normal cells, a normal range was
10 established of apparent allelic variation from 0.75 to 1.25 of the tumor/normal ratio. Therefore, only fluctuations in band intensity superior to this range were considered diagnostic of chromosomal imbalances. Even with 50% of normal tissue DNA contamination in tumor
15 DNA, the ratio of trisomy (in a hypothetical cell retaining diploidy in the rest of the chromosomes) would appear as 1.25 and the ratio of the loss of one allele would appear as 0.75. Our tumors did not have more than a 20-30% contamination of normal tissue, as analyzed by
20 histological examination (data not shown). Therefore, the approach used in the scoring of gains and losses has been conservative.

We claim:

1. A method of determining the clinical outcome of a subject with a cancer using a Genomic Damage Fraction comprising,

5 a. determining the relative change in quantity of nucleic acids between cancerous cells and non-cancerous cells of said subject;

b. determining the Genomic Damage Fraction from the results of step (a)

10 c. determining the prognosis of said subject according to said subject's GDF, where a GDF greater than a predetermined GDF is indicative of a first clinical outcome, and a GDF lesser than a predetermined GDF is indicative of a second clinical outcome.

15 2. The method of claim 1, wherein the relative change in quantity of nucleic acids is determined using AP-PCR DNA fingerprinting.

3. The method of claim 1, wherein said first clinical outcome is increased risk.

20 4. The method of claim 1, wherein said second clinical outcome is decreased risk.

5. The method of claim 1, wherein the relative change in nucleic acids is determined by the number of qualitative and/or quantitative changes in the
25 DNA fingerprint bands present in the cancerous cells as compared with the normal cells.

6. The method of claim 5, wherein the relative change in nucleic acids is determined by the number of quantitative changes in the DNA fingerprint
30 bands.

US 2011/0242426 A1

7. The method of claim 5, wherein the relative change in nucleic acids is determined by the number of qualitative changes in the DNA fingerprint bands.

5 8. The method of claim 5, wherein the relative change in nucleic acids is determined by the number of quantitative and qualitative changes in the DNA fingerprint bands.

9. The method of claim 1, wherein the
10 relative change in nucleic acids is a gain in quantity in nucleic acids.

10. The method of claim 1, wherein the relative change in nucleic acids is the combination of gain and loss in quantity in nucleic acids.

15 11. The method of claim 1, wherein the relative change in nucleic acids is a gain in quantity in nucleic acids.

12. The method of claim 1, wherein the subject with cancer has colorectal cancer.

20 13. A method of determining the clinical outcome of a subject with a cancer comprising,

a. generating the AP-PCR DNA fingerprint of non-cancerous cells from said subject;

b. generating the AP-PCR DNA fingerprint of
25 primary cancer cells from said subject;

c. generating the AP-PCR DNA fingerprint of metastatic cancer cells from said subject; and

6595363 "3436666

d. identifying chromosomal regions from AP-PCR DNA fingerprint data of steps (a), (b) and (c) wherein the occurrence of gains or losses of nucleic acids in certain chromosomal regions is prognostic of the clinical outcome for said subject.

14. The method of claim 13, wherein the gain and loss of nucleic acids is significantly different in metastatic cancer cells as compared to primary cancer cells.

15. The method of claim 13, wherein said chromosomal region is determined by a band of chromosome 4 obtained using the Blue primer (SEQ ID No: 1).

16. The method of claim 15, wherein said band is band N from the DNA fingerprint generated with the Blue primer (SEQ ID. NO:1).

17. A method of determining the clinical outcome of a subject with a cancer comprising,

a. generating the AP-PCR DNA fingerprint of non-cancerous cells from said subject;

b. generating the AP-PCR DNA fingerprint of primary cancer cells from said subject;

c. identifying chromosomal regions from AP-PCR DNA fingerprint data of steps (a) and (b), where gains or losses of nucleic acids occur; and

d. comparing said AP-PCR DNA fingerprints of chromosomes 1, 4, 6, 8, 9, and 13 from step a and step b wherein presence of gain or loss of nucleic acids in certain chromosomal regions is prognostic of the clinical outcome for said subject.

18. The method of claim 17 wherein said
chromosomal region that is determined by band N of
chromosome 4 from the BLUE primer (SEQ ID NO: 1)
fingerprint is prognostic of the clinical outcome for
5 said subject.

19. A method of predicting a clinical outcome
of a subject with cancer using an amplotype from said
subject comprising,

a. locating chromosomal regions that have
10 gained and lost nucleic acids using AP-PCR DNA
fingerprinting;

b. identifying said chromosomal regions that
have lost nucleic acids; and

c. identifying said chromosomal regions that
15 have gained nucleic acids;
wherein the combination of gains and losses according to
chromosomal regions are prognostic of the clinical
outcome for subject with cancer.

20. The method of claim 19, wherein the
20 results of step (b) and step (c) are displayed where said
gains and losses of nucleic acids are listed according to
the chromosomal regions where they occur, wherein the
combination of gains and losses according to chromosomal
regions are prognostic of the clinical outcome for
25 subject with cancer.

21. A method of identifying a genomic region
relevant for a cancer in a subject having said cancer
comprising,

(a) generating the AP-PCR DNA fingerprint of
30 non-cancerous cells, primary cancer, and metastatic tumor
cells from said subject; and

(b) identifying said genomic regions from
AP-PCR DNA fingerprint data of step (a), showing gains
and losses of nucleic acids in certain genomic regions
thereby identifying a genomic region linked to a cancer
5 gene.

22. The method of claim 21, wherein said
cancer is colorectal cancer.

23. The method of claim 21, wherein the said
AP-PCR DNA fingerprint is generated with the Blue primer
10 (SEQ ID NO: 1).

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69
70
71
72
73
74
75
76
77
78
79
80
81
82
83
84
85
86
87
88
89
90
91
92
93
94
95
96
97
98
99
100
101
102
103
104
105
106
107
108
109
110
111
112
113
114
115
116
117
118
119
120
121
122
123
124
125
126
127
128
129
130
131
132
133
134
135
136
137
138
139
140
141
142
143
144
145
146
147
148
149
150
151
152
153
154
155
156
157
158
159
160
161
162
163
164
165
166
167
168
169
170
171
172
173
174
175
176
177
178
179
180
181
182
183
184
185
186
187
188
189
190
191
192
193
194
195
196
197
198
199
200
201
202
203
204
205
206
207
208
209
210
211
212
213
214
215
216
217
218
219
220
221
222
223
224
225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246
247
248
249
250
251
252
253
254
255
256
257
258
259
260
261
262
263
264
265
266
267
268
269
270
271
272
273
274
275
276
277
278
279
280
281
282
283
284
285
286
287
288
289
290
291
292
293
294
295
296
297
298
299
300
301
302
303
304
305
306
307
308
309
310
311
312
313
314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341
342
343
344
345
346
347
348
349
350
351
352
353
354
355
356
357
358
359
360
361
362
363
364
365
366
367
368
369
370
371
372
373
374
375
376
377
378
379
380
381
382
383
384
385
386
387
388
389
390
391
392
393
394
395
396
397
398
399
400
401
402
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420
421
422
423
424
425
426
427
428
429
430
431
432
433
434
435
436
437
438
439
440
441
442
443
444
445
446
447
448
449
450
451
452
453
454
455
456
457
458
459
460
461
462
463
464
465
466
467
468
469
470
471
472
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493
494
495
496
497
498
499
500
501
502
503
504
505
506
507
508
509
510
511
512
513
514
515
516
517
518
519
520
521
522
523
524
525
526
527
528
529
530
531
532
533
534
535
536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551
552
553
554
555
556
557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577
578
579
580
581
582
583
584
585
586
587
588
589
590
591
592
593
594
595
596
597
598
599
600
601
602
603
604
605
606
607
608
609
610
611
612
613
614
615
616
617
618
619
620
621
622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640
641
642
643
644
645
646
647
648
649
650
651
652
653
654
655
656
657
658
659
660
661
662
663
664
665
666
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687
688
689
690
691
692
693
694
695
696
697
698
699
700
701
702
703
704
705
706
707
708
709
710
711
712
713
714
715
716
717
718
719
720
721
722
723
724
725
726
727
728
729
730
731
732
733
734
735
736
737
738
739
740
741
742
743
744
745
746
747
748
749
750
751
752
753
754
755
756
757
758
759
760
761
762
763
764
765
766
767
768
769
770
771
772
773
774
775
776
777
778
779
780
781
782
783
784
785
786
787
788
789
790
791
792
793
794
795
796
797
798
799
800
801
802
803
804
805
806
807
808
809
810
811
812
813
814
815
816
817
818
819
820
821
822
823
824
825
826
827
828
829
830
831
832
833
834
835
836
837
838
839
840
841
842
843
844
845
846
847
848
849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881
882
883
884
885
886
887
888
889
890
891
892
893
894
895
896
897
898
899
900
901
902
903
904
905
906
907
908
909
910
911
912
913
914
915
916
917
918
919
920
921
922
923
924
925
926
927
928
929
930
931
932
933
934
935
936
937
938
939
940
941
942
943
944
945
946
947
948
949
950
951
952
953
954
955
956
957
958
959
960
961
962
963
964
965
966
967
968
969
970
971
972
973
974
975
976
977
978
979
980
981
982
983
984
985
986
987
988
989
990
991
992
993
994
995
996
997
998
999
1000
1001
1002
1003
1004
1005
1006
1007
1008
1009
1010
1011
1012
1013
1014
1015
1016
1017
1018
1019
1020
1021
1022
1023
1024
1025
1026
1027
1028
1029
1030
1031
1032
1033
1034
1035
1036
1037
1038
1039
1040
1041
1042
1043
1044
1045
1046
1047
1048
1049
1050
1051
1052
1053
1054
1055
1056
1057
1058
1059
1060
1061
1062
1063
1064
1065
1066
1067
1068
1069
1070
1071
1072
1073
1074
1075
1076
1077
1078
1079
1080
1081
1082
1083
1084
1085
1086
1087
1088
1089
1090
1091
1092
1093
1094
1095
1096
1097
1098
1099
1100
1101
1102
1103
1104
1105
1106
1107
1108
1109
1110
1111
1112
1113
1114
1115
1116
1117
1118
1119
1120
1121
1122
1123
1124
1125
1126
1127
1128
1129
1130
1131
1132
1133
1134
1135
1136
1137
1138
1139
1140
1141
1142
1143
1144
1145
1146
1147
1148
1149
1150
1151
1152
1153
1154
1155
1156
1157
1158
1159
1160
1161
1162
1163
1164
1165
1166
1167
1168
1169
1170
1171
1172
1173
1174
1175
1176
1177
1178
1179
1180
1181
1182
1183
1184
1185
1186
1187
1188
1189
1190
1191
1192
1193
1194
1195
1196
1197
1198
1199
1200
1201
1202
1203
1204
1205
1206
1207
1208
1209
1210
1211
1212
1213
1214
1215
1216
1217
1218
1219
1220
1221
1222
1223
1224
1225
1226
1227
1228
1229
1230
1231
1232
1233
1234
1235
1236
1237
1238
1239
1240
1241
1242
1243
1244
1245
1246
1247
1248
1249
1250
1251
1252
1253
1254
1255
1256
1257
1258
1259
1260
1261
1262
1263
1264
1265
1266
1267
1268
1269
1270
1271
1272
1273
1274
1275
1276
1277
1278
1279
1280
1281
1282
1283
1284
1285
1286
1287
1288
1289
1290
1291
1292
1293
1294
1295
1296
1297
1298
1299
1300
1301
1302
1303
1304
1305
1306
1307
1308
1309
1310
1311
1312
1313
1314
1315
1316
1317
1318
1319
1320
1321
1322
1323
1324
1325
1326
1327
1328
1329
1330
1331
1332
1333
1334
1335
1336
1337
1338
1339
1340
1341
1342
1343
1344
1345
1346
1347
1348
1349
1350
1351
1352
1353
1354
1355
1356
1357
1358
1359
1360
1361
1362
1363
1364
1365
1366
1367
1368
1369
1370
1371
1372
1373
1374
1375
1376
1377
1378
1379
1380
1381
1382
1383
1384
1385
1386
1387
1388
1389
1390
1391
1392
1393
1394
1395
1396
1397
1398
1399
1400
1401
1402
1403
1404
1405
1406
1407
1408
1409
1410
1411
1412
1413
1414
1415
1416
1417
1418
1419
1420
1421
1422
1423
1424
1425
1426
1427
1428
1429
1430
1431
1432
1433
1434
1435
1436
1437
1438
1439
1440
1441
1442
1443
1444
1445
1446
1447
1448
1449
1450
1451
1452
1453
1454
1455
1456
1457
1458
1459
1460
1461
1462
1463
1464
1465
1466
1467
1468
1469
1470
1471
1472
1473
1474
1475
1476
1477
1478
1479
1480
1481
1482
1483
1484
1485
1486
1487
1488
1489
1490
1491
1492
1493
1494
1495
1496
1497
1498
1499
1500
1501
1502
1503
1504
1505
1506
1507
1508
1509
1510
1511
1512
1513
1514
1515
1516
1517
1518
1519
1520
1521
1522
1523
1524
1525
1526
1527
1528
1529
1530
1531
1532
1533
1534
1535
1536
1537
1538
1539
1540
1541
1542
1543
1544
1545
1546
1547
1548
1549
1550
1551
1552
1553
1554
1555
1556
1557
1558
1559
1560
1561
1562
1563
1564
1565
1566
1567
1568
1569
1570
1571
1572
1573
1574
1575
1576
1577
1578
1579
1580
1581
1582
1583
1584
1585
1586
1587
1588
1589
1590
1591
1592
1593
1594
1595
1596
1597
1598
1599
1600
1601
1602
1603
1604
1605
1606
1607
1608
1609
1610
1611
1612
1613
1614
1615
1616
1617
1618
1619
1620
1621
1622
1623
1624
1625
1626
1627
1628
1629
1630
1631
1632
1633
1634
1635
1636
1637
1638
1639
1640
1641
1642
1643
1644
1645
1646
1647
1648
1649
1650
1651
1652
1653
1654
1655
1656
1657
1658
1659
1660
1661
1662
1663
1664
1665
1666
1667
1668
1669
1670
1671
1672
1673
1674
1675
1676
1677
1678
1679
1680
1681
1682
1683
1684
1685
1686
1687
1688
1689
1690
1691
1692
1693
1694
1695
1696
1697
1698
1699
1700
1701
1702
1703
1704
1705
1706
1707
1708
1709
1710
1711
1712
1713
1714
1715
1716
1717
1718
1719
1720
1721
1722
1723
1724
1725
1726
1727
1728
1729
1730
1731
1732
1733
1734
1735
1736
1737
1738
1739
1740
1741
1742
1743
1744
1745
1746
1747
1748
1749
1750
1751
1752
1753
1754
1755
1756
1757
1758
1759
1760
1761
1762
1763
1764
1765
1766
1767
1768
1769
1770
1771
1772
1773
1774
1775
1776
1777
1778
1779
1780
1781
1782
1783
1784
1785
1786
1787
1788
1789
1790
1791
1792
1793
1794
1795
1796
1797
1798
1799
1800
1801
1802
1803
1804
1805
1806
1807
1808
1809
1810
1811
1812
1813
1814
1815
1816
1817
1818
1819
1820
1821
1822
1823
1824
1825
1826
1827
1828
1829
1830
1831
1832
1833
1834
1835
1836
1837
1838
1839
1840
1841
1842
1843
1844
1845
1846
1847
1848
1849
1850
1851
1852
1853
1854
1855
1856
1857
1858
1859
1860
1861
1862
1863
1864
1865
1866
1867
1868
1869
1870
1871
1872
1873
1874
1875
1876
1877
1878
1879
1880
1881
1882
1883
1884
1885
1886
1887
1888
1889
1890
1891
1892
1893
1894
1895
1896
1897
1898
1899
1900
1901
1902
1903
1904
1905
1906
1907
1908
1909
1910
1911
1912
1913
1914
1915
1916
1917
1918
1919
1920
1921
1922
1923
1924
1925
1926
1927
1928
1929
1930
1931
1932
1933
1934
1935
1936
1937
1938
1939
1940
1941
1942
1943
1944
1945
1946
1947
1948
1949
1950
1951
1952
1953
1954
1955
1956
1957
1958
1959
1960
1961
1962
1963
1964
1965
1966
1967
1968
1969
1970
1971
1972
1973
1974
1975
1976
1977
1978
1979
1980
1981
1982
1983
1984
1985
1986
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021
2022
2023
2024
2025
2026
2027
2028
2029
2030
2031
2032
2033
2034
2035
2036
2037
2038
2039
2040
2041
2042
2043
2044
2045
2046
2047
2048
2049
2050
2051
2052
2053
2054
2055
2056
2057
2058
2059
2060
2061
2062
2063
2064
2065
2066
2067
2068
2069
2070
2071
2072
2073
2074
2075
2076
2077
2078
2079
2080
2081
2082
2083
2084
2085
2086
2087
2088
2089
2090
2091
2092
2093
2094
2095
2096
2097
2098
2099
2100
2101
2102
2103
2104
2105
2106
2107
2108
2109
2110
2111
2112
2113
2114
2115
2116
2117
2118
2119
2120
2121
2122
2123
2124
2125
2126
2127
2128
2129
2130
2131
2132
2133
2134
2135
2136
2137
2138
2139
2140
2141
2142
2143
2144
2145
2146
2147
2148
2149
2150
2151
2152
2153
2154
2155
2156
2157
2158
2159
2160
2161
2162
2163
2164
2165
2166
2167
2168
2169
2170
2171
2172
2173
2174
2175
2176
2177
2178
2179
2180
2181
2182
2183
2184
2185
2186
2187
2188
2189
2190
2191
2192
2193
2194
2195
2196
2197
2198
2199
2200
2201
2202
2203
2204
2205
2206
2207
2208
2209
221

ABSTRACT OF THE INVENTION

A method of determining the clinical outcome of a subject with a cancer using a Genomic Damage Fraction comprising, (a) determining the relative change in
5 quantity of nucleic acids between cancerous cells and non-cancerous cells of said subject, (b) determining the Genomic Damage Fraction from the results of step (a), and (c) determining the prognosis of said subject according to said subject's GDF, where a GDF greater than a
10 predetermined GDF is indicative of a first clinical outcome (e.g., a poor prognosis), and a GDF lesser than a predetermined GDF is indicative of a second clinical outcome (e.g., a good prognosis); and a method of identifying certain genomic sequences whose alterations
15 during tumorigenesis of a subject with a cancer have prognostic value for determining the clinical outcome of said subject comprising, (a) determining the molecular profiles of genomic losses and gains ("amplotyping") of tumors at different stages of progression from the same
20 cancer patient, (b) identifying changes (losses and gains) specifically associated to the more advanced stages of tumor progression (e.g., metastatic stage), and (c) determining the prognosis of said subject according to said subject's status of these genomic sequences of
25 step b, where a change (loss or gain) is indicative of a first clinical outcome (i.e., poor prognosis), and no change (i.e., no loss or gain) is indicative of a second clinical outcome (i.e., good prognosis).

"SECRET"

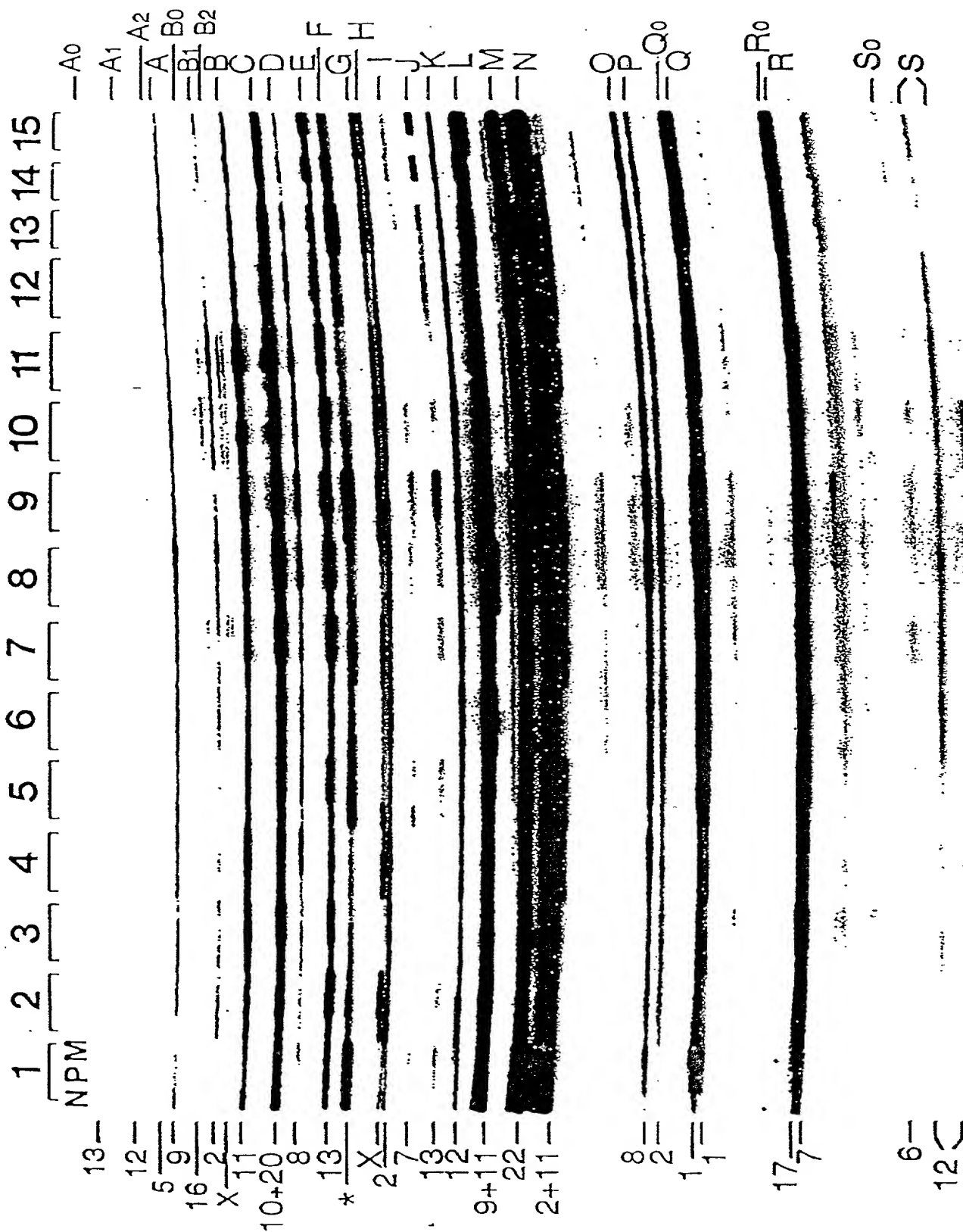


FIGURE 1

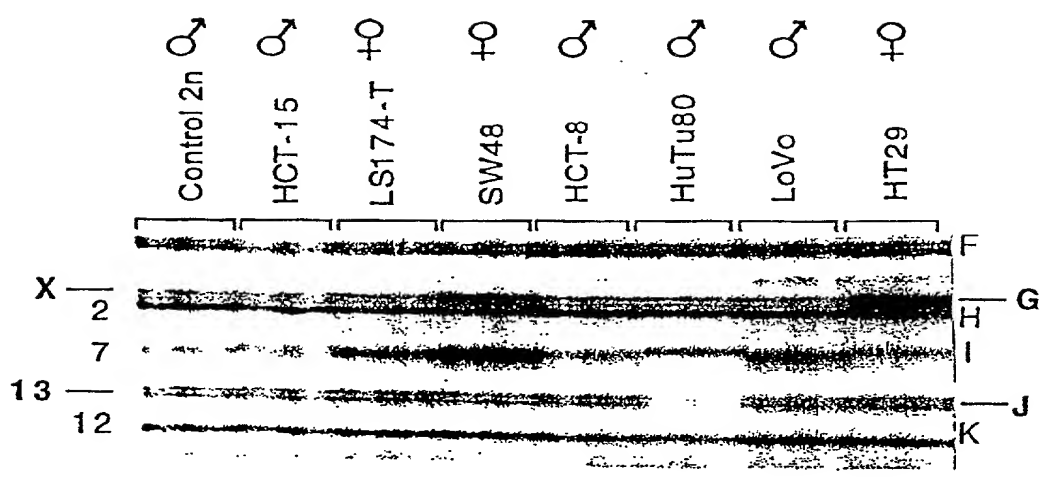


FIGURE 2

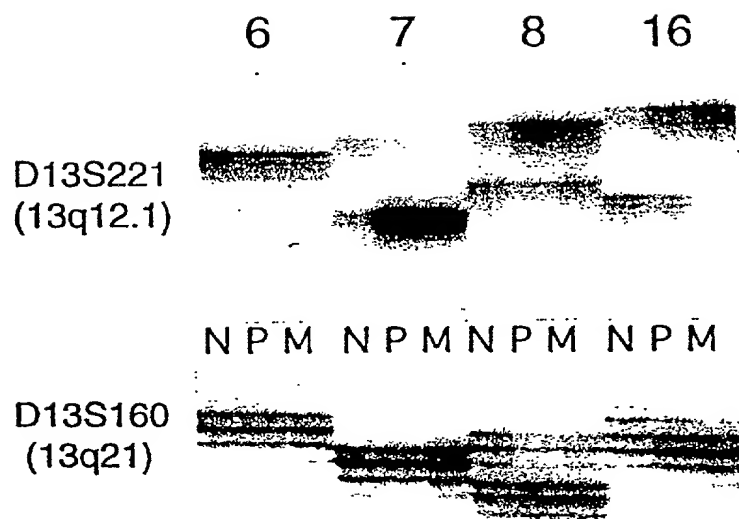


FIGURE 3

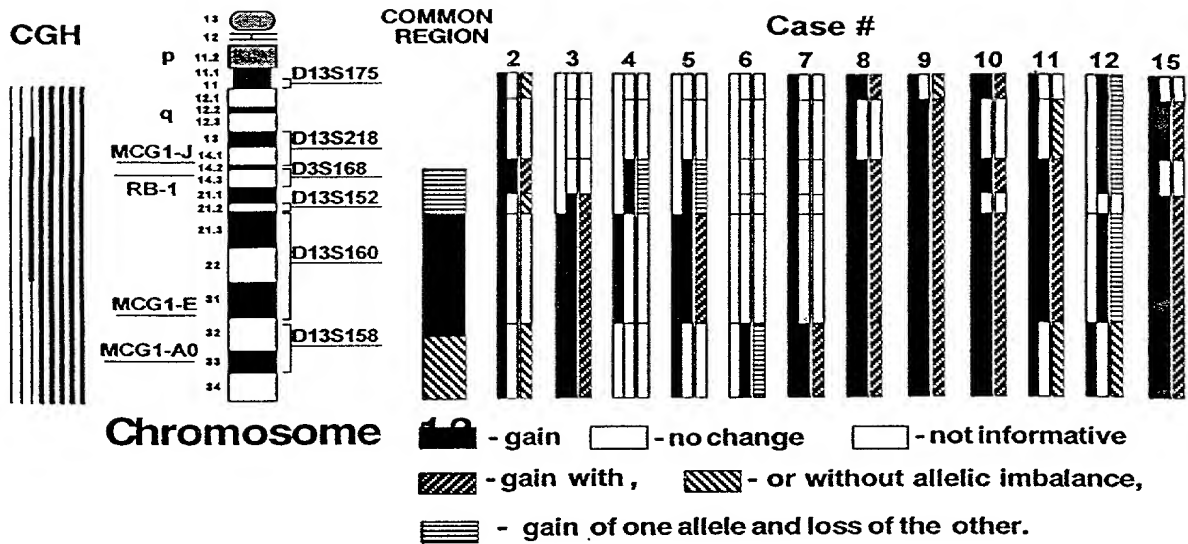


FIGURE 4

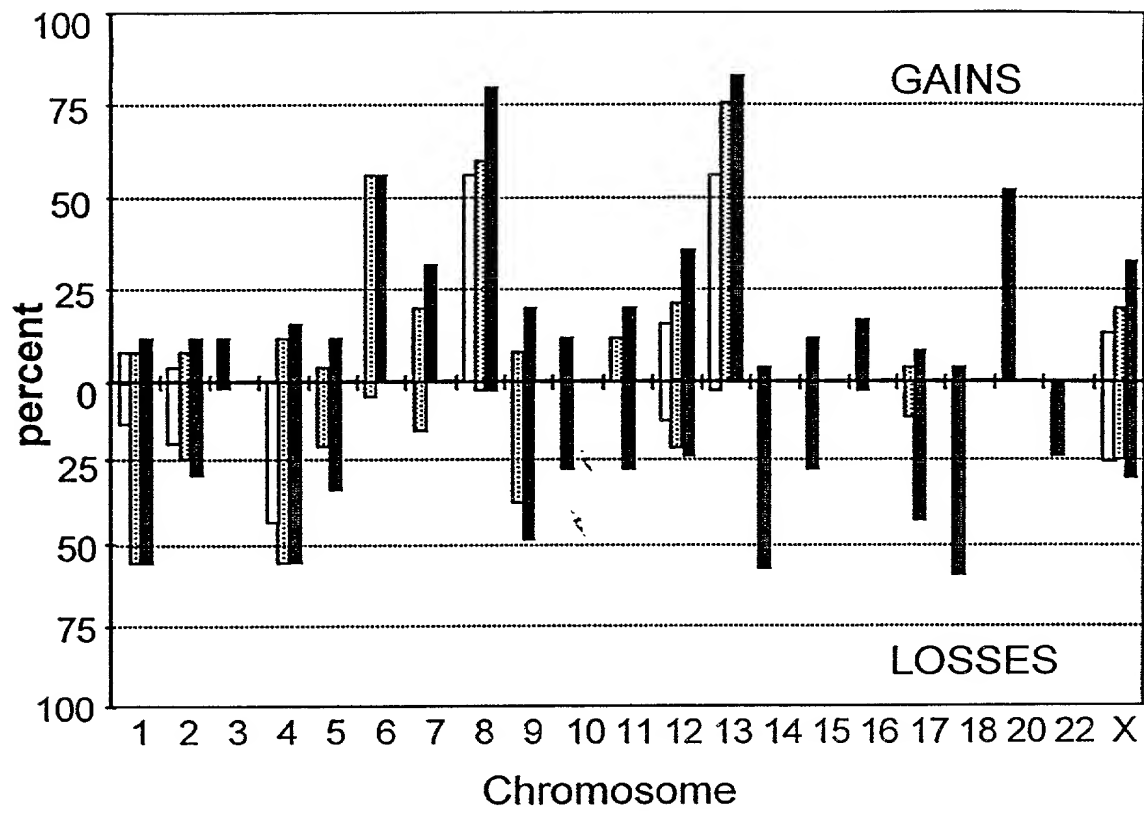


FIGURE 5

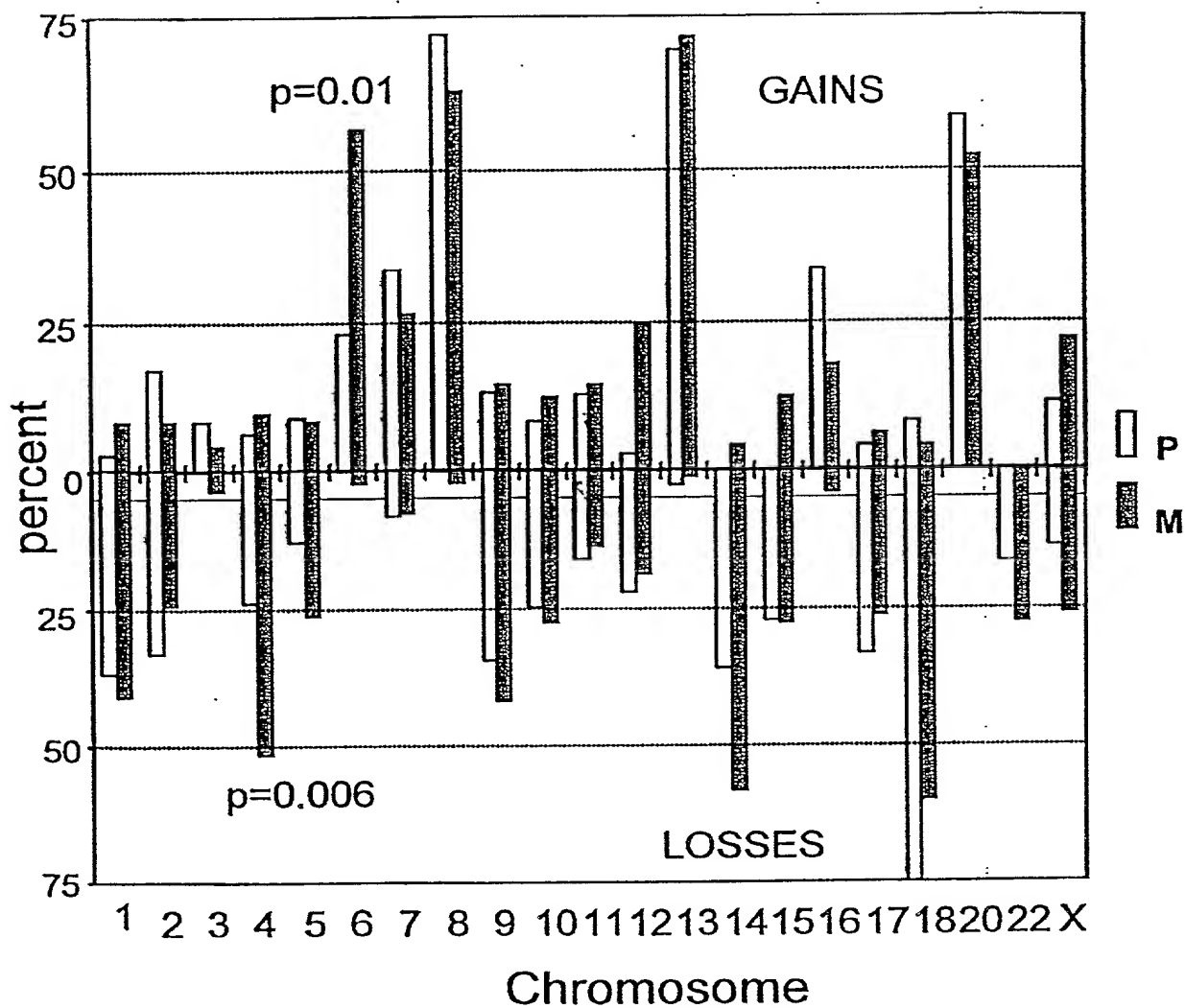


FIGURE 6

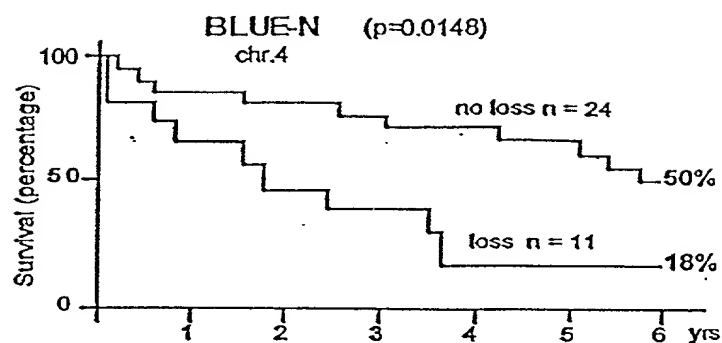


FIGURE 7